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Chemistry

Experimental evidence for secondary protein–chromophore interactions at the Schiff base linkage in bacteriorhodopsin: Molecular mechanism for proton pumping

(resonance Raman spectroscopy/isotopically labeled purple membrane/pH titration of M_{412} kinetics/active transport/proton switch-ion gate molecular regulation)

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ABSTRACT **Resonance Raman spectroscopy of the retinylidene chromophore in various isotopically labeled membrane environments together with spectra of isotopically labeled model compounds demonstrates that a secondary protein interaction is present at the protonated Schiff base linkage in bacteriorhodopsin. The data indicate that although the interaction is present in all protonated bacteriorhodopsin species it is absent in unprotonated intermediates. Furthermore, kinetic resonance Raman spectroscopy has been used to monitor the dynamics of Schiff base deprotonation as a function of pH. All our results are consistent with lysine as the interacting group. A structure for the interaction is proposed in which the interacting protein group in an unprotonated configuration is complexed through the Schiff base proton to the Schiff base nitrogen. These data suggest a molecular mechanism for proton pumping and ion gate molecular regulation. In this mechanism, light causes electron redistribution in the retinylidene chromophore, which results in the deprotonation of an amino acid side chain with pK >10.2 ± 0.3 (e.g., arginine). This induces subsequent retinal and protein conformational transitions which eventually lower the pK of the Schiff base complex from >12 before light absorption to 10.2 ± 0.3 in microseconds after photon absorption. Finally, in this low pK state the complex can reprotonate the proton-deficient high pK group generated by light, and the complex is then reprotonated from the opposite side of the membrane.**

The discovery of the purple membrane in *Halobacterium halobium* (1) has generated considerable interest in the field of bioenergetics. It has been shown that, when this bacterium is grown under low oxygen tension and in the light, purple membrane patches develop in the plasma membrane (1). These purple membrane patches contain a two-dimensional crystalline array (2) of a single protein that has been called "bacteriorhodopsin" (1) because of its apparent similarities with visual pigments. These similarities include retinal-based chromophores covalently bound via Schiff base linkages to the ϵ-amino group of lysine residues and similar red shifts and subsequent blue shifts in their absorption spectra as a result of chromophore photon absorption. However, unlike visual photoreceptor rhodopsins, which contain the 11-*cis* isomer of the chromophore and are quantum detectors, bacteriorhodopsin containing the all-*trans* isomer is an energy converter that acts as a light-driven proton pump (3–5) coupled to ATP synthesis (4, 5) in support of the Mitchell chemosmotic hypothesis (6). Thus, the molecular mechanism underlying the function of this membrane-bound proton pump is a problem of fundamental importance in biology.

In recent years, resonance Raman spectroscopy has become an important tool for monitoring the environmentally and structurally sensitive vibrational spectra of chromophoric sites in macromolecular complexes (7, 8). The application of this technique to bacteriorhodopsin (bR_{570}) has already demonstrated that the Schiff base is protonated and can be deuterated (9). Furthermore, steady-state measurements with this technique have also shown that the Schiff base is deprotonated during the proton pumping cycle by the M_{412} intermediate (9) [between bR_{570} and M_{412} there are at least two intermediates, K and L (10)], and kinetic resonance Raman spectroscopy has demonstrated that this deprotonation occurs at pH 7 on a time scale of 10–30 μsec (11). When this rate of Schiff base deprotonation is contrasted to the formation time of K [the intermediate produced in picoseconds by bacteriorhodopsin (bR_{570}) photon absorption (12, 13)], it becomes evident that the retinylidene chromophore is involved not only in the primary mechanism of excitation but also in the subsequent proton pumping function of this membrane.

In this paper we use steady-state and kinetic resonance Raman spectroscopy on native bacteriorhodopsin, isotopically labeled bacteriorhodopsin, and model all-*trans* retinal (H-ret) Schiff bases to demonstrate that a secondary protein–chromophore interaction is present at the Schiff base linkage. Furthermore, we present data on the dynamics of the deprotonation of the Schiff base as a function of pH. These data clearly indicate that a protein residue of pK 10.2 ± 0.3 is controlling the rate of deprotonation of the Schiff base. Finally, we integrate the above results into a model for the protein–Schiff base complex that suggests a plausible scheme for the molecular mechanism of proton pumping.

MATERIALS AND METHODS

H. halobium (S_9) was grown by standard procedures (14) and bacteriorhodopsin was purifed and isolated by the methods of Kanner and Racker (15). Fully deuterated bacteriorhodopsin and ^{15}N-enriched bacteriorhodopsin were grown at Argonne National Laboratories by described procedures.‡ Resonance Raman measurements indicated that the bacteriorhodopsin was >95% isotopically substituted. Apomembrane (H-opsin) and deuterated apomembrane (D-opsin) were prepared by the method of Oesterhelt *et al.* (16). H-ret was purchased from Eastman Kodak Company and used without further purification. Fully deuterated all-*trans* retinal (D-ret) was isolated from fully deuterated bacteriorhodopsin by the method of Pettei *et al.* (17). Reconstitutions (18, 19) were performed to prepare the following four systems: (*i*) H-ret + H-opsin; (*ii*) H-ret + D-opsin; (*iii*) D-ret + H-opsin; and (*iv*) D-ret + D-opsin.

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Abbreviations: H-opsin, apomembrane; D-opsin, deuterated apomembrane; H-ret, all-*trans* retinal; D-ret, fully deuterated all-*trans* retinal.

‡ Crespi, H. (1977) *Proceedings of the IAEA/CMEA, TECH. Committee, Meeting on Modern Trends in the Biological Application of Stable Isotopes*, Leipzig, Austria.

For comparison, protonated and unprotonated Schiff bases of H-ret with butylamine, [^{2}H]butylamine, methylamine, [^{15}N]methylamine, and a methylated Schiff base of butylamine were prepared by procedures reported elsewhere (20). Resonance Raman spectra of these model systems were obtained by using 10 mW of 476.2 nm excitation and the Raman spectrometer and flow apparatus described by Marcus and Lewis (11). Spectra of the isotopically labeled membranes were recorded with the same spectrometer and under steady-state conditions, with 40 mW of 457.9 nm excitation. Spectral resolution in all cases was 2–3 cm^{-1}.

To obtain the pH dependence of the kinetics of Schiff base deprotonation and M_{412} production, bacteriorhodopsin suspensions were excited with 50 mW of 457.9 nm laser light and were flowed at various rates through a capillary tube of known diameter, as described (21). The pH of the suspensions was varied and the data were analyzed as discussed in Fig. 3.

RESULTS AND DISCUSSION

An outstanding feature of the bacteriorhodopsin resonance Raman spectrum is that the C=N⊕H stretching mode at 1642 cm^{-1} (9) is significantly (~13 cm^{-1}) lower in frequency than any of the simple protonated Schiff bases of H-ret. In addition, Marcus *et al.* (19), Aton *et al.* (22), and Terner *et al.* (23) have pointed out that the resonance Raman spectrum of the protonated Schiff base species bR_{570} in the structurally sensitive fingerprint region (1100–1400 cm^{-1}) is not modeled well by a simple all-*trans* protonated Schiff base of butylamine. This is the case even though all biochemical extraction procedures indicate that the chromophore in bR_{570} is in all-*trans* conformation (17). On the other hand, visual pigments have C=N⊕H stretching frequencies at 1655 cm^{-1}, and, in agreement with biochemical extraction data, Sulkes *et al.* (24) (for squid acid metarhodopsin) and Mathies *et al.* (25) (for bovine rhodopsin and isorhodopsin) have demonstrated that these pigments are modeled well by simple protonated Schiff bases of all-*trans*, 11-*cis*, and 9-*cis* retinal, respectively. The above results may suggest that the protonated Schiff base in bacteriorhodopsin is altered by an environmental interaction that also perturbs the fingerprint vibrational spectrum of the all-*trans* chromophore.

Evidence for Secondary Protein–Schiff Base Interactions in bR_{570} and Other Protonated Bacteriorhodopsin Species. To test the above hypothesis we used resonance Raman spectroscopy to study a series of bacteriorhodopsin samples with isotopically altered chromophore or protein environments. As shown in Fig. 1 *A* and *B*, the C=N⊕H stretching frequency in H_2O is lowered in frequency by 7 cm^{-1} upon deuteration of all the nonexchangeable protons on the protein (bacterio-opsin). This is especially significant in view of the fact that vibrational modes in other regions of the resonance Raman spectrum of bacteriorhodopsin are unaltered upon changing the isotopic nature of the protein (19). Furthermore, a similar lowering in the C=N⊕H stretching frequency is also detected when fully deuterated retinal is incorporated into protonated and deuterated bacterio-opsin suspended in H_2O (Fig. 1 *D* and *E*). It can be readily demonstrated that this effect does not arise from fully deuterating the lysine side chain covalently attached to the Schiff base. This is evident if one compares the frequency of the C=N⊕H vibration in all-*trans* protonated Schiff bases of butylamine and fully deuterated butylamine (20). Butylamine has essentially the same number of protons as the lysine side chain, and the frequency of the C=N⊕H stretching vibration is identical (1655 cm^{-1}) in both these molecules. On the other hand, it can be shown (20) that, although mass changes in the butyl group do not affect the carbon–nitrogen stretching frequency, altering the character of the Schiff base nitrogen

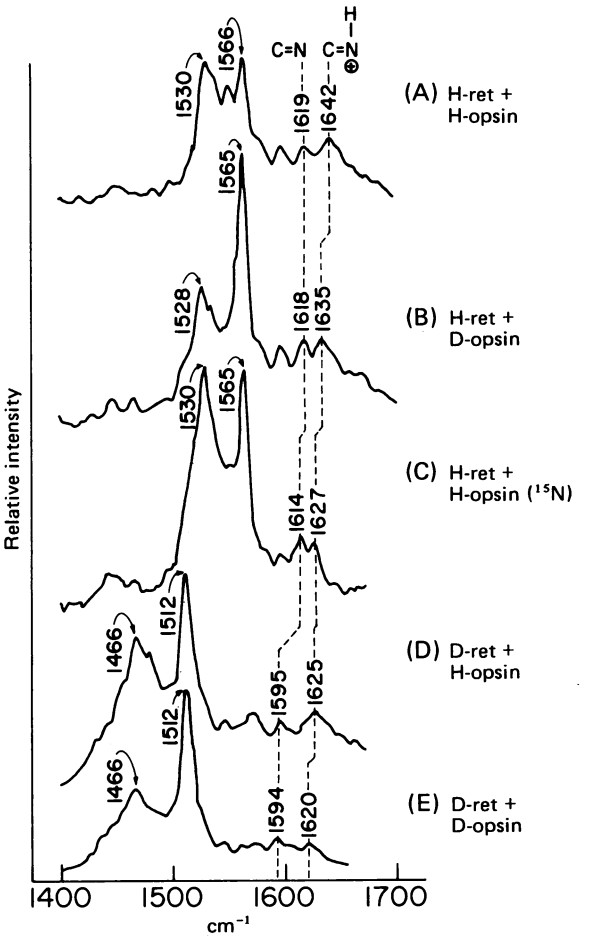

FIG. 1. Steady-state resonance Raman spectra in H_2O (pH 6.6) of H-ret + H-opsin (native bacteriorhodopsin) (*A*), H-ret + D-opsin (*B*), ^{15}N-enriched bacteriorhodopsin (H-ret + H-opsin) (*C*), D-ret + H-opsin (*D*), and D-ret + D-opsin (fully deuterated bacteriorhodopsin) (*E*), all obtained with 40 mW of 457.9 nm laser excitation. Resolution is 2–3 cm^{-1} in each case. The 1530 and 1566 cm^{-1} bands are assigned to the C=C stretches of bR_{570} and M_{412} respectively (see *A–C*). The intense bands at 1466 and 1512 cm^{-1} are assigned to the C=C stretches of bR_{570} and M_{412} in bacteriorhodopsin samples containing fully deuterated retinal (unpublished data).

substituent (such as the replacement of the proton by a methyl group) does lower the frequency of this vibrational mode significantly to 1630 cm^{-1}. Therefore, the above data indicate that in addition to the covalent lysine linkage there is a group interacting at the Schiff base with some nonexchangeable protons.

Lack of Schiff Base Interaction in Unprotonated Species. Fig. 1 *A* and *D* shows that the unprotonated species, M_{412}, has a —C=N— stretching frequency at ~1619 cm^{-1} (H-ret) and ~1595 cm^{-1} (D-ret) that is essentially unaffected by deuterating its protein environment. This further supports the deduction from the model compound data on protonated Schiff bases that the carbon–nitrogen stretching frequency is unaltered upon deuteration of the lysine side chain. Thus, these data indicate that the deprotonation of the Schiff base removes the secondary protein–Schiff base interaction. This suggests that the Schiff base proton is the link that establishes the secondary interaction between the bacterio-opsin matrix and the Schiff base.

Nature of the Interacting Protein Group. By comparing the data in Fig. 1 *A* and *C*, it can be seen that ^{15}N enrichment of bacterio-opsin lowers the frequency of the unprotonated Schiff base —C=N— stretching vibration from 1619 cm^{-1} to 1614 cm^{-1} (5 cm^{-1}). On the other hand, the effect of ^{15}N enrichment

on the C=N⊕H stretching frequency is significantly larger. A 15-cm^{-1} shift is observed from 1642 cm^{-1} in fully protonated bacteriorhodopsin to 1627 cm^{-1} in ^{15}N-enriched bacteriorhodopsin. Thus, protonated species in which a secondary protein–Schiff base interaction exists exhibit an effect of ^{15}N enrichment that is 3 times the magnitude observed for unprotonated Schiff base species lacking this interaction. However, the spectra of the ^{15}N and ^{14}N model compounds do not show different isotopic effects for the protonated and unprotonated Schiff bases (20). Therefore, these data indicate but do not prove that the interacting group has a nitrogen in contact with the Schiff base proton.

Identification of the Interacting Group. Besides the amide group of the peptide backbone there are only five amino acid residues containing nitrogen in their side chains: arginine, asparagine, glutamine, histidine, and lysine. In order to differentiate among all these groups (side chains and backbone), we measured the rate of deprotonation of the Schiff base as a function of pH by using kinetic resonance Raman spectroscopy. These data clearly show that the rate of Schiff base deprotonation increases drastically as the pH is increased. The titration curves for two different vibrational modes shown in Fig. 2 demonstrate that there is a group *directly* controlling the rate of Schiff base deprotonation with a pK of 10.2 ± 0.3. Of the above mentioned residues, only the ϵ-amino group of lysine has a pK (10.5) in this vicinity, suggesting that lysine may be the interacting group. In this regard it should be noted that we cannot rule out the possibility that the species responsible for the interaction is a phospholipid with a molecular structure complexed to the phosphate head group mimicking a lysine side chain (e.g., 3′-*O*-lysylphosphatidylglycerol). However, the reported lipid composition of the purple membrane (26) does not indicate the presence of any such lipids. Therefore, it is likely that a secondary lysine residue is interacting with the Schiff base proton. Furthermore, the published peptide sequence (27, 28) in the vicinity of the Schiff base linkage,

retinylidene
|
-Asp-Ala-Lys-Lys-Phe-Tyr-Ala-,

may indicate that the lysine adjacent to the Schiff base linkage is involved in this interaction. However, until a detailed structural map of bacteriorhodopsin is available it will not be known whether the interacting amino acid is in the above peptide sequence or is removed in sequence but close in space to the Schiff base.

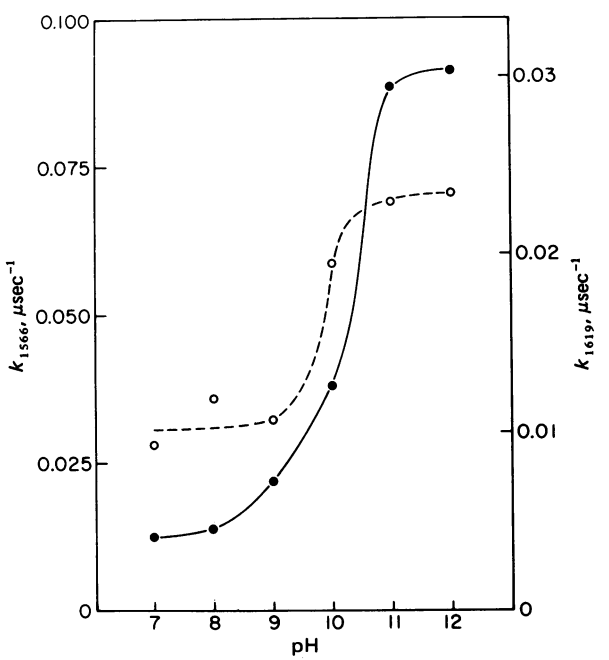

FIG. 2. pH titration curves of the formation rate constants of the unprotonated Schiff-base stretching frequency k_{1619} (O - - - O) and of the M_{412} species C=C stretching frequency k_{1566} (● — ●). The Raman intensities of the bands at 1642 (C=N⊕H stretching frequency), 1619 (C=N stretching frequency), 1530 (bR_{570} C=C stretch), and 1566 cm^{-1} (M_{412} C=C stretch) were measured at different transit times in the laser beam. The intensity ratios 1619/1642 and 1566/1530 were plotted against time and the initial slopes of these plots give k_{1619} and k_{1566}, respectively. This was performed at pH values from 7 to 12, and the titration curves in this figure exhibit inflection points at 9.9 ± 0.25 for k_{1619} and 10.35 ± 0.1 for k_{1566}. Because of the stronger Raman bands at 1530 and 1566 cm^{-1}, the statistical quality of the k_{1566} titration curve is probably better than that of the k_{1619} titration curve.

Although all our data indicate that a secondary lysine residue is indeed complexed to the Schiff base proton, two points of caution should be noted. First, in spite of the ^{15}N data, we cannot exclude the possibility of an interaction with a tyrosine side chain that also has a pK around 10. However, we have chemically modified (nitrated) tyrosine (unpublished results) and, even though such a modification alters the absorption spectrum, the C=N⊕H stretching frequency is completely unaltered. This suggests that protein–chromophore interactions in addition to the one discussed above are present. Second, pKs of amino acids buried in proteins are known in certain instances to differ from the value observed for the free amino acid. However, it should be noted that the Schiff base is accessible to aqueous solvent (9) and thus the group interacting with the Schiff base is probably not in a hydrophobic environment.

Proposed Structure of the Interaction. In order to determine the structure of the Schiff base proton–protein complex, other experimental observations have to be considered. First, the interaction is clearly present in the initial bacteriorhodopsin species (bR_{570}) even at pH values well above 10.2 ± 0.3, the pK of Schiff base deprotonation microseconds after light absorption. This is demonstrated by the unaltered C=N⊕H stretching frequency in bR_{570} up to pH 12 (21). Second, the interacting Schiff base proton can be exchanged for a deuteron from the external medium (9). These results taken together indicate that, even though the immediate environment of the noncovalently linked amino acid residue is accessible to the external medium, the Schiff base proton–protein interaction cannot be altered by titration to pH 12 without light. Thus, assuming for the sake of discussion that lysine is the interacting residue, we propose the structure shown in Fig. 3*A* for the complex in which the lysine ϵ-amino group is maintained in an NH_2 configuration. In this complex the simple protonated Schiff base is altered by an interaction with the unpaired electrons of the lysine ϵ-amino group. Such an interaction is by no means unique to this protein structure. It has previously been proposed in other systems—for example, in the deamination of α-methylaspartic acid by the enzyme aspartate aminotransferase (29). It should also be noted that a structure similar to the one depicted in Fig. 3*A* in which the Schiff base proton protects the interacting amino acid side chain from deprotonation could be drawn for a number of other amino acids including tyrosine and arginine.

The above suggestion readily accounts for the anomalously low frequency of the C=N⊕H stretch in bacteriorhodopsin. Furthermore, the spectra we have obtained (20) for a methylated Schiff base of H-ret demonstrate that altering the character of the Schiff base substituent not only lowers the carbon–nitrogen stretching frequency but also alters the all-*trans* retinylidene Schiff base fingerprint structure which was previously thought to be specific to the isomeric state of the chromophore. Therefore, it is not surprising that attempts to model

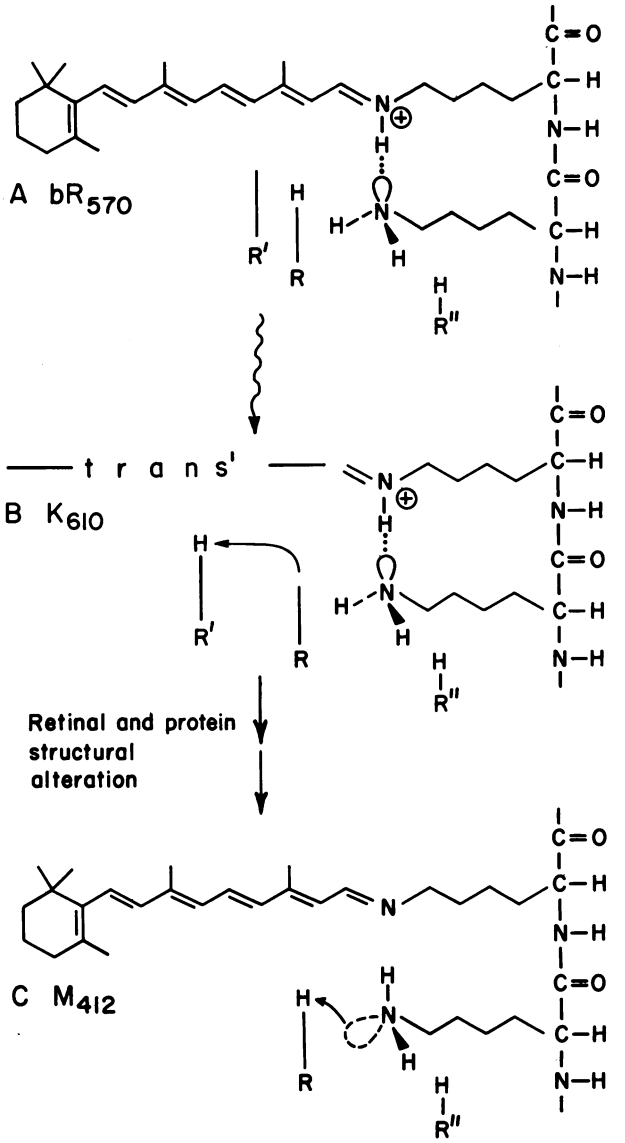

FIG. 3. (*A*) Proposed structure for the secondary protein–Schiff base interaction in bacteriorhodopsin. (*B*) Plausible molecular transformation in the protein for the photon-induced bR_{570}-to-K_{610} transition that can readily initiate proton pumping and can be stabilized by retinal structural alteration depicted as "trans' " in this figure. (*C*) Depiction of Schiff base deprotonation and dissociation of the protein–Schiff base complex.

the bacteriorhodopsin bR_{570} chromophore structure with a simple protonated Schiff base of H-ret have failed. Our data indicate that such modeling attempts with all-*trans* chromophores will be successful when a molecular complex is synthesized to represent accurately all of the protein–chromophore interactions in bacteriorhodopsin's active site.

MODEL FOR PROTON PUMPING

Our proposed structure for bacteriorhodopsin's active site, when coupled to other experimental observations, suggests a plausible molecular mechanism for proton pumping. These observations are the following. (*i*) Kinetic resonance Raman spectroscopy of the C=N⊕H stretching frequency indicates that, as long as the Schiff base is protonated, an interaction similar to the one we have proposed is present (ref. 11; unpublished results), (*ii*) deprotonation of the Schiff base removes the interaction, and (*iii*) Schiff base deprotonation occurs rapidly (in μseconds) at physiological pH (11) even though the pK of the Schiff base complex prior to deprotonation has been lowered from >12 to only 10.2 ± 0.3 (21). This last observation leads us to conclude that, for such complex deprotonation and M_{412} formation to occur at physiological pH and even at pH values as low as 3, a group with pK > 10.2 ± 0.3 lacking a proton must in all probability be prepared in the initial events that excite this proton pump.

The above observations and conclusion suggest a molecular mechanism of proton pumping (see Fig. 3) in which the Schiff base complex (with a pK that has been lowered from >12 to 10.2 ± 0.3 by thermally driven chromophore and protein structural alterations) is deprotonated from one side of the membrane by a proton-deficient high pK group represented as R in Fig. 3 and is reprotonated from the other side of the membrane by a group depicted as R″ in Fig. 3. Although we cannot exclude the possibility of other protein or lipid components being involved, the most likely candidates for R and R″ are arginine and tyrosine, respectively. Our choice of tyrosine is based on chemical modification experiments (see above) and the pH dependence of bR_{570} re-formation, whereas arginine, with pK ~ 12.5, was chosen because it would be stable to solvent-induced deprotonation in the pH range 3–12 where photochemical cycling associated with proton pumping occurs.

Let us now consider what light-induced molecular transformations can result in a high pK group such as arginine lacking a proton at pH values between 3.0 and 12. Such a molecular transformation requires a large perturbation to the system, and photon absorption is certainly the most likely source of this perturbation. One possibility is that photon-induced electron redistribution in retinal could perturb (for example, electrostatically) the electron density of protein residues intimately associated with this chromophore, thus facilitating the proton transfer strongly suggested by our experiments.

Further support for an excitation mechanism in which light causes proton transfer in the protein can be found by studying the purple membrane below pH 3.0. At pH 2.5, photochemical cycling is seriously inhibited and bacteriorhodopsin's absorption red shifts to 605nm (1), a value close to the absorption of the primary photochemical intermediate K_{610} (10). This last result suggests that the photochemically produced red shift may be due to protonation of a group with a low pK (e.g., aspartic acid). Thus, we propose that the primary action of light in bacteriorhodopsin is to translocate a proton from R to R′ (Fig. 3) (likely, although by no means exclusive, candidates for these groups are arginine and aspartic acid, respectively), a translocation that would not normally occur without energy from photon absorption.

This proposal supports the suggestion that energy is stored in the primary photochemical process (30–32). A simple expression relating free energy to the pK of the groups involved in the above proton translocation gives approximately 12 kcal of energy stored in K_{610}. This agrees well with the value 13 kcal calculated by Rosenfeld *et al.* (30) from kinetic data. Furthermore, the decay of K_{610} to a blue-shifted pigment absorption similar to that of bR_{570} occurs in 2 μsec, and Eigen and Schoen (33) have shown that acetate groups, in fact, dissociate in 1 μsec.

Such a mechanism of light-induced proton translocation in the protein stabilized by retinal structural alteration has been recently proposed by Lewis (32) and results in an energy surface that accounts for all the presently available photophysical and photochemical data on bacteriorhodopsin. In this mechanism, electron redistribution in the vertically excited state of the retinal chromophore results in a proton translocation in the protein in picoseconds. The feasibility of this mechanism is also supported by the recently observed (34) deuterium isotope effect on the formation of K_{610} because resonance Raman spectroscopy indicates that the Schiff base (which contains the

only exchangeable proton on the chromophore) changes its state of protonation not in picoseconds but only on a microsecond timescale. In addition, the results presented in this paper further support the interpretation (32) of a similar deuterium isotope effect on the photochemistry of bovine rhodopsin (35). As we have noted, there is no evidence in resonance Raman spectra of photoreceptor rhodopsins that would indicate the presence of a secondary protein–Schiff base proton interaction similar to the one we have observed in bacteriorhodopsin. In fact, all aspects of the resonance Raman spectra of bovine and squid rhodopsin are modeled well by simple protonated Schiff bases of retinal (24, 25). Thus, the primary photochemical transformations in both rhodopsin and bacteriorhodopsin that produce remarkably similar red shifts on similar time scales with different chromophore conformations and Schiff base interactions must involve some region in the active site removed from the Schiff base and unrelated to a simple 11-*cis*-to-*trans* chromophore isomerization. A proton translocation in the protein certainly fits all of the above observations.

SUMMARY

We have demonstrated in this paper that a protein residue (probably the ϵ-amino group of lysine) is interacting with the Schiff base proton in all protonated bacteriorhodopsin species. This interaction is absent in unprotonated species. We have also constructed a model for this molecular interaction with the amino acid deprotonated and the Schiff base protonated. In addition, we have demonstrated that, microseconds after light absorption, the pK of the Schiff base complex is altered from >12 in bR_{570} to 10.2 ± 0.3. Furthermore, in order to account for photochemical cycling in which the complex deprotonates rapidly [initial rate of M_{412} formation (k_{1566}) is ~ 75 μsec at pH 6] and the Schiff base reprotonates slowly (milliseconds), one has to postulate a distinct source of protons that reprotonate the Schiff base, thus reforming the complex. A likely candidate for this source of protons is tyrosine. Therefore, all our data support a mechanism in which a series of groups translocate protons.

Our molecular mechanism for proton pumping and ion gate molecular regulation in bacteriorhodopsin can be summarized as follows. A photon induces electron redistribution in the vertically excited state of the retinylidene chromophore (36, 37), resulting (see Fig. 3 *A* and *B*) in a proton translocation from R to R′ (32) and an alteration in retinal structure that separates these groups, preventing the return of the proton to R. We have argued that likely candidates for the above protein residues are arginine and aspartic acid, respectively. Subsequently, R′ releases its proton in ~ 2 μsec, probably to a sequence of proton-translocating groups such as R_1-H ... R_2-H ... R_3-H that eventually leads the proton out one side of the membrane. [The theory of such proton-translocating groups has been discussed by Nagle and Morowitz (38).] The unstable protein structure with R deprotonated drives further retinal and protein conformational changes that subsequently alter the pK of the Schiff base complex to ~ 10.2. Thus, the Schiff base complex can now be deprotonated and can reprotonate (see Fig. 3*C*) the high pK group represented as R. The Schiff base is then reprotonated directly or indirectly from the opposite side of the membrane by group R″ to re-form the protein–protonated Schiff base complex. An interesting aspect of the above model is that Schiff base pK alteration occurs in the thermal steps of the proton pumping cycle. This suggestion may indicate that additional protons per photon could be pumped if the pK of Schiff bases in neighboring bacteriorhodopsin molecules, which did not absorb light, were altered allosterically and became active participants in proton-conducting chains.

Finally, this molecular mechanism of electron redistribution followed by proton translocation may also apply to other membrane-bound ion pumps such as those proteins involved in photosynthesis and electron transport.

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